AssayMax™

Corticosterone (CORT) ELISA Kit

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For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Assay Summary

Step 1. Add 25 µl of Standard or Sample and 25 µl of Biotinylated Protein per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of SP Conjugate per well.
Incubate 30 minutes.

Step 3. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 20 minutes.

Step 4. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Symbol Key

Consult instructions for use.

Assay Template

	A	B	C	D	E	F	G	H
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Corticosterone (CORT) ELISA Kit

Catalog No. EC3001-1

Sample insert for reference use only

Introduction

Corticosterone is the adrenal steroid, the major glucocorticoid. Glucocorticoid hormones are also known as corticosteroid hormones and are synthesized mainly in the adrenal cortex; however, more recently the enzymes involved in their synthesis have been found in a variety of cells and tissues, including the heart. The effects of these hormones are mediated via both cytoplasmic mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which act as ligand-inducible transcription factor (1). Corticosterone has a profound effect on the structure and function of the hippocampus (2, 3). Brain corticosterone action through the glucocorticoid receptor may involve memory storage (4). Emotional stress might cause increases in plasma corticosterone (5).

Principle of the Assay

The AssayMax Corticosterone ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of corticosterone in **plasma, serum, urine, milk, saliva, and cell culture samples**. This assay employs a quantitative **competitive enzyme immunoassay** technique that measures corticosterone in less than 3 hours. A polyclonal antibody specific for corticosterone has been pre-coated onto a 96-well microplate with removable strips. Corticosterone in standards and samples is competed with a biotinylated corticosterone sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is Not For Use In Diagnostic Procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated protein, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.

- Spin down the SP conjugate vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Corticosterone Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against corticosterone.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Corticosterone Standard:** Corticosterone in a buffered protein base (100 ng/ml, 0.5 ml).
- **Biotinylated Corticosterone:** 1 vial, lyophilized.
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (20 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate, 100x)**: A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard and SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Biotinylated Protein at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute human plasma 1:4 into EIA Diluent or within the range of 1x-20x, and assay. Dilute rat or mouse plasma 1:100 into EIA Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute human serum 1:4 into EIA Diluent or within the range of 1x-20x, and assay. Dilute rat or mouse serum 1:100 into EIA Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute human urine 1:10, rat urine 1:20, and mouse urine 1:20 into EIA Diluent, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Saliva:** Collect human saliva using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Milk:** Collect human milk using sample tube. Centrifuge samples at 800 x *g* for 10 minutes and assay. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Refer to Sample Dilution Guidelines below for further instruction.

Guidelines for Dilutions of 1:100 or Greater (*for reference only; please follow the insert for specific dilution suggested)*	
1:100	**1:10000**
A) 4 ul sample: 396 µl buffer(100x) = 100 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000 fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1:1000	**1:100000**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000 fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Allow the standard to warm to room temperature prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (100 ng/ml) 1:4 with EIA Diluent to produce 25, 6.25, 1.563, and 0.391 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Corticosterone] (ng/ml)
P1	1 part Standard (100 ng/ml)	100.0
P2	1 part P1 + 3 parts EIA Diluent	25.00
P3	1 part P2 + 3 parts EIA Diluent	6.250
P4	1 part P3 + 3 parts EIA Diluent	1.563
P5	1 part P4 + 3 parts EIA Diluent	0.391
P6	EIA Diluent	0.000

- **Biotinylated Corticosterone (3x):** Reconstitute Biotinylated Corticosterone with 4 ml EIA Diluent to produce a 3-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to

making dilutions. The stock solution should be further diluted 1:3 with EIA Diluent. Any remaining solution should be frozen at -20°C and used within 30 days.

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 μl of Corticosterone Standard and/or sample per well, and immediately add 25 μl of Biotinylated Corticosterone to each well (on top of the standard or sample) and tap plate to mix gently. Cover wells with a sealing tape and incubate for 2 hours at room temperature. Start the timer after the last addition.
- Wash five times with 200 μl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 μl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 μl of Streptavidin-Peroxidase conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for 20 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at low concentration points

after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

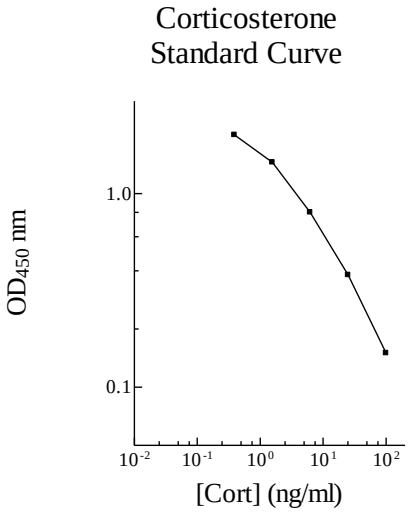
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	ng/ml	OD	Average OD
P1	100.0	0.123 0.127	0.125
P2	25.00	0.263 0.252	0.257
P3	6.250	0.550 0.526	0.538
P4	1.563	0.959 0.936	0.948
P5	0.391	1.244 1.240	1.242
P6	0.000	1.800 1.698	1.749
Sample: Human Normal, Sodium Citrate Plasma (4x)		0.784 0.778	0.781

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- The normal human plasma levels of corticosterone are 2 – 22 ng/ml.
- Human plasma and serum samples from healthy adults were tested (n=40). On average, corticosterone level was 9.4 ng/ml.

Sample	n	Average Value (ng/ml)
Human Pool Normal Plasma	10	7.6
Human Normal Plasma	20	9.2
Human Pool Normal Serum	10	11.5

Performance Characteristics

- The minimum detectable dose of corticosterone as calculated by 2SD from the mean of a zero standard was established to be 0.28 ng/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.2%	4.9%	4.3%	10.3%	9.7%	10.5%
Average CV (%)	4.1%			10.2%		

Spiking Recovery

- Recovery was determined by spiking two plasma samples with different corticosterone concentrations.

Sample	Unspiked Sample (ng/ml)	Spike (ng/ml)	Expected	Observed	Recovery (%)
1	1.0	50	51.00	54.6	107%
		12.5	13.50	14.21	105%
		3.125	4.125	4.026	98%
2	10.0	50	60.00	58.11	97%
		12.5	22.50	21.68	96%
		3.125	13.125	13.48	103%
Average Recovery (%)					101%

Linearity

- Plasma and serum samples were serially-diluted to test for linearity.

Average Percentage of Expected Value (%)		
Sample Dilution	Human Plasma	Human Serum
1:2	90%	92%
1:4	100%	99%
1:8	97%	102%

Cross-Reactivity

Species	Cross Reactivity (%)
Beagle	<50%
Bovine	None
Monkey	<50%
Mouse	100%
Rat	100%
Swine	100%
Rabbit	100%

Human	100%
Name	**Cross Reactivity (%)**
PROGESTERONE	<2%
ALLOPREGNANOLONE	<0.1%
CORTEXOLONE	<1%
DEOXYCORTICOSTERONE	<30%
CORTISONE	None
CORTEXOLONE HEMISUCCINATE	None
CORTICOSTERONE	100%
6-KETO-17β-ESTRADIOL	None
5-ANDROSTEN-3β-OL-7, 17-DIONE	None
6-KETO-17α-ESTRADIOL	None
3-KETO-5α, 16-ANDROSTENE	None
4-ANDROSTEN-17α-OL-3-ONE	None
ALDOSTERONE	<2%
ETHYNYLESTRADIOL	None
6-KETOESTRIOL	None
6-KETOESTRONE	None
17β-HYDROXY-4-ANDROSTENE-3, 11-DIONE	<0.1%
CORTISONE Acetate	None
ALDOSTERONE 21-HEMISUCCINATE	<0.3%
4-PREGNEN-17, 20β- DIOL-3-ONE	<0.2%
11α-HYDROXYTESTOSTERONE	None
20α-HYDROXYPROGESTERONE	None
6β-HYDROXYPROGESTERONE	<0.1%
HYDROCORTISONE	None
17-HYDROXYPROGESTERONE	<0.1%
CORTISOL	<0.1%

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are dry after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.

	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

References

(1) Sheppard KE. (2003) Vitam *Horm* 66:77-112
(2) Schaaf MJ *et. al.* (2000) *Stress* 3(3):201-8
(3) Herbert J. (1998) *Exp Gerontol* 33(7-8): 713-27
(4) Sandi C. (1998) *Neural Plast* 6(3): 41-52
(5) Tanaka M. (1999) *Ind Health* 37(2): 143-56